Leptin and Weight, part 2

In this lecture we go deeper into understanding the weight setpoint and how it is affected by interventions. We will write down the full simple model and compare it to experiments on rats. The goal is to see the beauty and utility of a math model for answering questions of substance, like the effects of food quality and exercise on fat mass.

nice deep sigh of relief

A glimpse into how we work, with models and experimental data
The content of these lectures on weight is research from my lab that is not published yet- you are the first to see it. A world premiere:) It started with PhD student Omer Karin, and the torch was taken up by PhD student Alon Bar, who got inspired to compare the model to data from rats. This is how we do research on physiology- write minimal models, compare them to a century of experiments that were usually done for other reasons. We also compare to large medical datasets, as we will see in upcoming lectures. When possible, we test the theory with new experiments.

Alon Bar considered the feeding experiments of Ruth Harris, Thomas Kasser and Roy Martin (1986) . The experimenters aimed to find out the body composition (fat, proteins) when feeding changes. Their temporal data is so precise it can be reused for our purposes here. When rats were put on 40% of their normal diet for a few weeks, they lost fat mass (Fig. 1). This restrictive diet was then stopped, and the rats were allowed to eat ad-libitum (freely). At first they ate more than normal (overshoot), and every day ate less and less until they returned to their normal fat and food intake.

Conversely, when overfed by tube feeding at 160% of their normal food intake, they fattened (Fig. 2). After tube feeding was stopped, the rats ate less than normal (undershoot) and gradually returned to their normal weight and food intake.

The experiment thus has two parts: forced feeding, and then recovery. The forced feeding part can be used to test and calibrate the diet line. The recovery part maps onto the appetite line.

We can get the diet line from noting the steady state fat in the different conditions. Let's make a phase portrait of food intake u versus fat F. Rats restricted to 40% of normal food intake (u=6g/day) end up with almost zero fat. This is one point on the diet line. Rats overfed to 160% their normal intake reached fat of about 2.5 times higher than normal. This is another point on the diet line. It looks pretty much like a straight line as expected.

The appetite line can be derived from the recovery dynamics. After the starvation condition is stopped, mice overshoot to eat about 20g per day, about 30% higher than their normal intake of 15g/d. They then slowly trace out a line in the phase portrait as they lose fat and eat
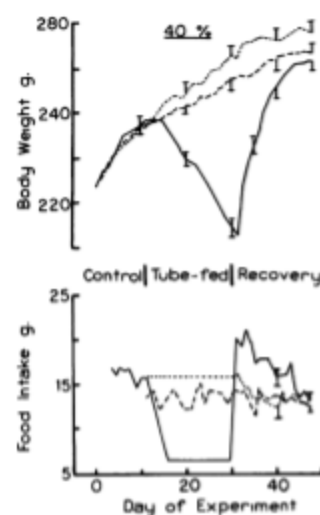

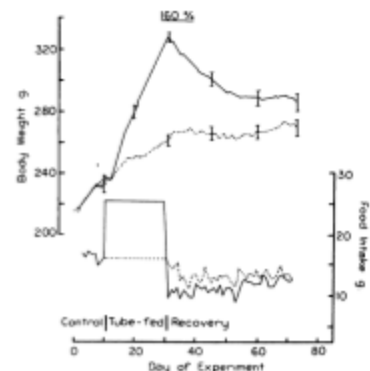Fig. 1 Rats weight and intake dynamics at 40% food restriction

Fig. 2 Rats weight and intake dynamics at 160% food overfeeding

less, until approaching the normal level. After the overfeeding condition, they eat less, about 10g/day. They drop rapidly in fat but keep eating about the same, which gives the nullcline a concave shape that drops vertically in this region, before converging back to the setpoint. We gain a nice experimental picture of the diet and appetite lines (Fig. 3).
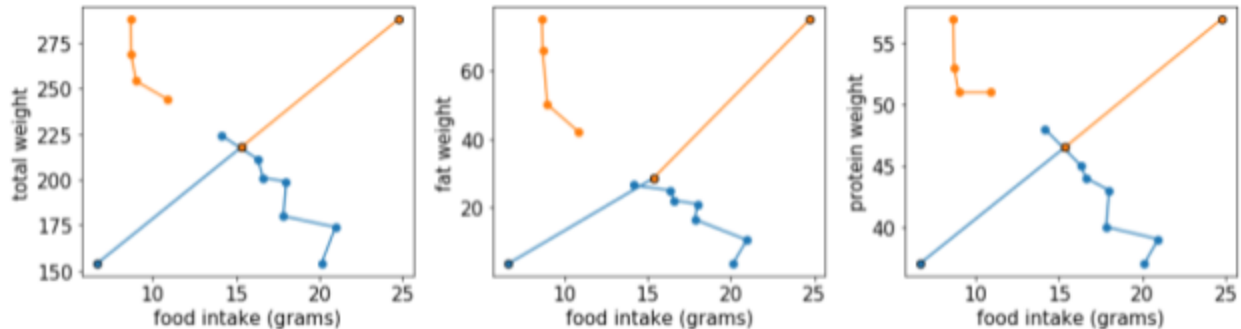

Fig. 3 experimental construction of the diet and appetite lines

Equations for fat determine the rate of dieting

We now shift to writing equations for these lines. The idea is to demonstrate how these equations help us answer new questions, like what is the effect of changes in food quality or exercise.

In lecture 1 we wrote down an equation for the rate of change of fat, basically a conservation equation for bioenergetic balance: fat changes due to food intake, metabolic costs, and the cost of fat itself:

Rate of fat change = (change from food) - (change from metabolic costs) - (change from cost of fat)

Which in math language is

$$(1)\ dF/dt = \alpha_F u - \gamma_E - \gamma_F\, F$$

This equation has three parameters. The parameter α_F is the conversion factor (or 'exchange rate') of a gram of food to a gram of fat, with units of [gr Fat/gr food]. The second parameter γ_E is the amount of fat needed to supply the energy cost of the body over a day, in units of [gr fat/time]. The rate of fat loss due to the energy cost of fat itself is γ_F in units of [1/time]. For reference, note that the energy stored in fat is about 9 kilocalories per gram.

When food intake u is constant, as forced by the experimenters, we can solve this equation. Suppose u is kept low for a while. The solution is an exponential decline of weight to a new steady-state.

The new steady-state is found by setting dF/dt=0, because steady-state is, by definition, the level of F where it stops changing. We obtain from Eq 1: $dF/dt = 0 = \alpha_F u - \gamma_E - \gamma_F F$. Solving this yields the diet line:

$$(2)\ F_{st} = \alpha_F/\gamma_F\, u - \gamma_E/\gamma_F$$

This is steady-state fat when u is constant.

nice deep sigh of relief

How quickly does fat reach its steady state? We can fully solve Eq (1) over time. This is a solution of an ordinary linear differential equation, and is thus always of the form

$$F(t) = Ae^{-\gamma_F t} + B$$

To make sure this is really a solution, we take the time derivative dF/dt, to find Eq 1 back again. We can determine A and B by making sure that $F(t)$ starts at its initial condition $F(0)$ at $t = 0$, and ends up at F_{st} at infinite time. To do so, note that at $t \to \infty$, the exponent goes to zero $e^{-\gamma_F t} \to 0$, so that $B = F_{st}$. When t=0 the exponent is $e^{-\gamma_F 0} = 1$ and thus $A = F(0) - F_{st}$. We obtain therefore:

$$(3)\ F(t) = (F_{st} - F(0))(1 - e^{-\gamma_F t}) + F(0)$$

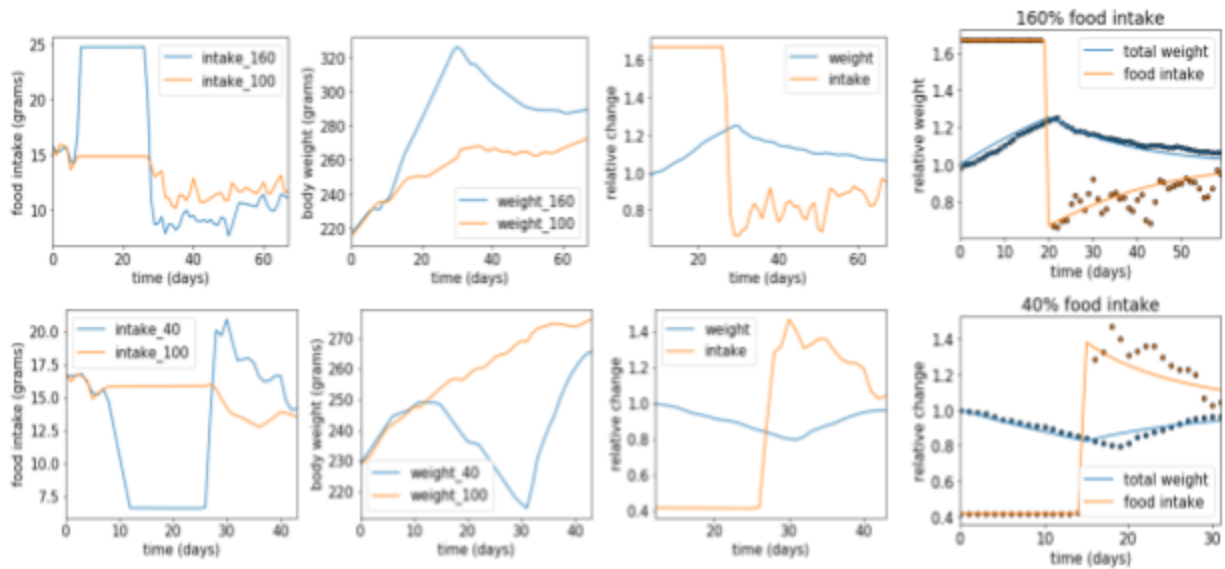This solution compares well with the experiments of Harris et al (Fig. 4).

Fig. 4 Daily food intake and weight dynamics of rats constrained to 40% or 160% daily food intake. Data is normalize to relative change from control group.

From this comparison we can find the rate at which fat changes- how long do I need to diet before I get halfway to the steady state? The half-life for fat, as always in a differential equation like this, is determined by the constant that multiplies F, namely γ_F. The γ_F parameter has units of $1/time$, and indeed the half-life which has units of time is proportional to $1/\gamma_F$. To find it precisely, we need to find when $e^{-\gamma_F t_{1/2}} = 1/2$, which, when taking log of both sides, results in:

$$(4)\ t_{1/2} = ln(2)/\gamma_F \quad \textbf{fat half-life}$$

Since the fat half-life depends only on γ_F, and not on the initial or final fat levels, we can see that the half-way time from one steady state to another steady state is always the same. This applies to loss or gain of fat.

Our differential equation, Eq 1, describes the rat data very well (Fig. 5). The timescale for changes in fat shows a half-life in rats of about 10 days, giving $\gamma_F = 0.07\ d^{-1}$.
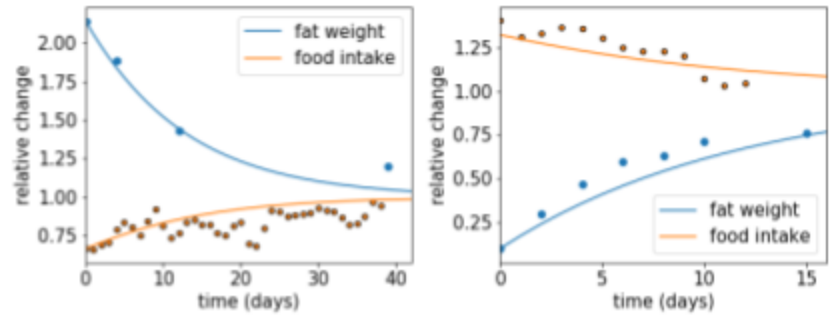

Fig. 5. Fat recovery to steady state dynamics

Mathematical model for the appetite line
Lets next consider the appetite line. This is slightly harder than the diet line, but hopefully we will be fine.
nice deep sigh of relief

OK. Appetite is controlled by leptin (Fig. 6). Leptin is made by fat cells, and its discovery elevated fat from the prosaic status of a fuel tank and thermal insulator, to a smart tissue that uses hormones to talk with the brain and other organs. Leptin, L, is secreted by fat in the presence of food intake[1] . For example, in starvation for a day or two, less leptin is secreted by a given amount of fat than during a fed state, which is a great way to make the animal eat more when it is starved. Since leptin production rate grows with both fat and with food intake, it can be modeled as a product of fat mass F times food intake u , with a rate
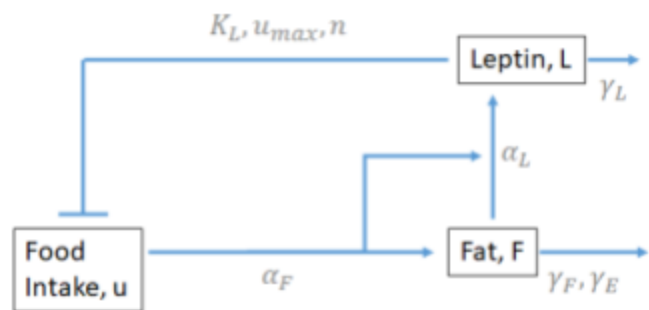

Fig. 6 Leptin hormone circuit controls food intake and weight

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[1] How food intake controls leptin secretion is unclear. For experts: it seems not to be due to post-meal rise in insulin, but instead to be more related to average insulin over a few days.

parameter α_L placed in front: $\alpha_L u \cdot F$. Leptin is removed by clearance in the kidney, which gives each molecule of leptin a removal rate γ_L, making a total removal of $\gamma_L L$ molecules per unit time. This is the kind of removal processes we will use throughout the course: like a radioactive particle that has a certain probability per unit time to decay, so each molecule has a probability per unit time to be removed, described by the removal rate. The difference between production and removal gives an equation for the rate of change of leptin:

$$(5)\ dL/dt = \alpha_L u \cdot F - \gamma_L L$$

The removal of leptin is rapid, with a half-life of about 40 minutes. As always, leptin half-life is determined by the removal parameter γ_L, so that $ln(2)/\gamma_L \sim 40min$. Leptin dynamics are thus much faster than the fat dynamics which change over many days. We can thus assume that leptin is at steady-state, $dL/dt = 0$, which is another use of the principle of separation of timescales. Plugging in dL/dt=0 to Eq 5, we find

$$(6)\ L = \alpha_L u \cdot F/\gamma_L \ .$$

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Now we are ready for the appetite line. Food intake is suppressed by leptin, as we saw. This inhibition has a halfway effect when leptin concentration is K_L. Thus, the appetite, defined as the food intake over a day given ad-libitum conditions, can be written as a decreasing function of leptin $u = f(L/K_L)$.

We can be more concrete by giving a specific form to the function f. We use an excellent biochemical model for the effect of a hormone when binding to a receptor. This is the **Hill function** (Fig. 7), derived in Appendix A, where:

Fig. 7 leptin control of food intake is described by hill function

$$(8)\ u/u_{max} = 1/(1 + (L/K_L)^n)$$

The half-way point is K_L, and the steepness is determined by the Hill coefficient n. In this function, when there is no leptin, eating is at its maximal "satiety" value, u_{max}. This maximal satiety is due to stomach distention, hormones like *ghrelin* and *glp1*, and other factors. Leptin decreases appetite: The more leptin, the less appetite.

To get the appetite line, we use steady-state leptin for a given fat and food level from Eq 6, namely $L = \alpha_L u \cdot F/\gamma_L$, and plug in the leptin level L that provides food intake u deduced from inverting Eq 8, $L = K_L (u_{max}/u - 1)^{1/n}$, to obtain the appetite line:

$$(9)\ F = \frac{K_L \gamma_L}{\alpha_L u} (u_{max}/u - 1)^{1/n} \quad \textit{the appetite line}$$

The appetite line is a decreasing function of u as expected: the more fat - the less food intake. It intersects the x-axis at the maximal food intake u_{max}. The appetite line curves up at high fat and has a distinctive concave shape. The higher the leptin resistance K_L, the more this curve shifts to the right, pivoting around u_{max}. This explains why I drew the appetite line the way I did in the first lecture.

The appetite line has a parameter $K_L \gamma_L/\alpha_L$ which is a combination of leptin production and removal rates and letpin resistance.
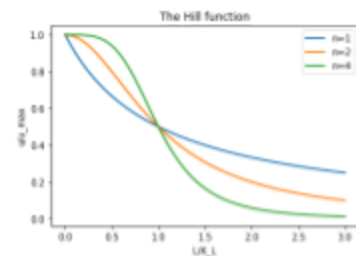
With the two nullclines in hand, we can compare the model to the experiments on rats when they recover from over- and under-feeding (Fig. 8). The experimental data shows behavior that is similar to the model. The diet line rises linearly and intersects the x-axis at a certain intake rate. The appetite line drops in a curved way.
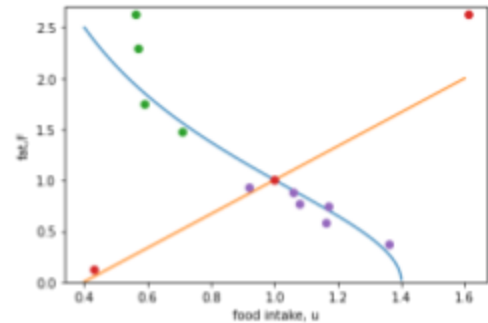

Fig. 8 Rat data compares well with diet and appetite lines

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Normalized variables help to reduce the number of free parameters
If we use the rat data and set the normal rat food intake and fat both to 1, we can have a model with fewer parameters.
The diet nullcline is a straight line that intersects the x axis at u_0 =0.4 and goes through (1,1), and thus is

$$(10)\ F = (u - u_0)/(1 - u_0)$$

The appetite nullcline intersects the x axis at u_{max} =1.4, and thus

$$(11)\ F = (u_{max}/u - 1)^{1/n}/u(u_{max} - 1)^{1/n}$$

These scaled nullclines agree with the rat data, with one free parameter, the Hill coefficient n of leptin action. A value of n=2 gives reasonable agreement.

Difference in weight setpoint between individuals:
Importantly, since different individuals have different parameters, the appetite line and the diet line differ from individual to individual. In humans, such parameters vary with age, especially in children and after age 50. Our lifestyles, including food quality and exercise levels, also vary. As a result, we each have our own weight set point. The model can now help us evaluate the effects of different parameters and different interventions (Fig. 9). The effects are clearly seen when we draw arrows around the set point indicating the effect of changing each parameter.

Two parameters increase both fat and weight: increase in leptin resistance K_L and in the satiety level u_{max} . These parameters shift only the appetite line.
The rest of the parameters shift both diet and appetite lines. Increasing food 'fatness', α_F , the parameter which determines the rate at which food is converted to fat, causes a large rise in fat and a small drop in food intake. This is the solution to the puzzle at the end of lecture 1.
Increasing exercise or metabolic rate raises γ_E , which causes a reduction in fat and an increase in food intake; the relative increase
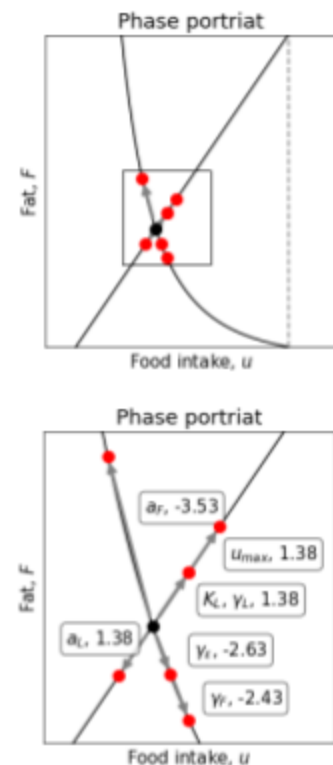

Fig. 9 Analyzing the effect of each parameter on the steady state

in intake is smaller than the relative increase in fat. This agrees with experiments in which rodents are given a wheel, which lowers fat by 30% and increases food intake by 20%. The major parameters that increase weight setpoint are thus: food fatness, satiety, reduced metabolic rate, leptin resistance.

Fig. 10 The relation between leptin and fat across the population is quadratic

Differences in leptin between people: We can go from rodents to humans for a moment, even though the model is not guaranteed to apply precisely. In humans, leptin varies widely between people, and so does percent fat. In fact, leptin goes approximately as percent fat squared, $L{\sim}F^2$ (Fig. 10). In mice as well, mutants with a dysfunctional leptin receptor (db/db mice) have 250% more fat and 6 times more leptin, matching the square dependence since $2.5^2{\sim}6$. This square dependence seems to contradict a step in our thinking, where we said that leptin goes proportional to fat, not fat squared (Eq 3). This proportionality applies, however, for a given individual with a given set of parameters: twice the fat, twice the leptin.
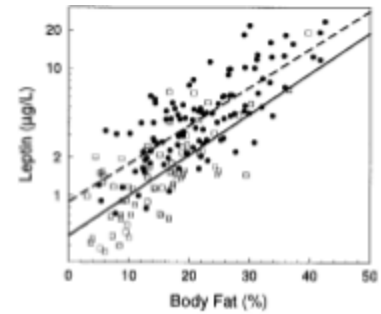
When comparing different individuals, we need to remember they have different parameter sets. It turns out that variation in one of the model parameters can give the square relation between leptin and fat (Fig. 11). This parameter is u_{max}, the satiety point, the maximal food intake. It is predicted to be a major cause for the difference between individual leptin levels. Other factors such as exercise, food quality and basal metabolic rate have important but smaller effects. Thus, treatments that lower u_{max}, such as GLP1 hormone that causes satiety, or surgical treatments such as gastric bypass, are expected to have a large effect on the weight setpoint.

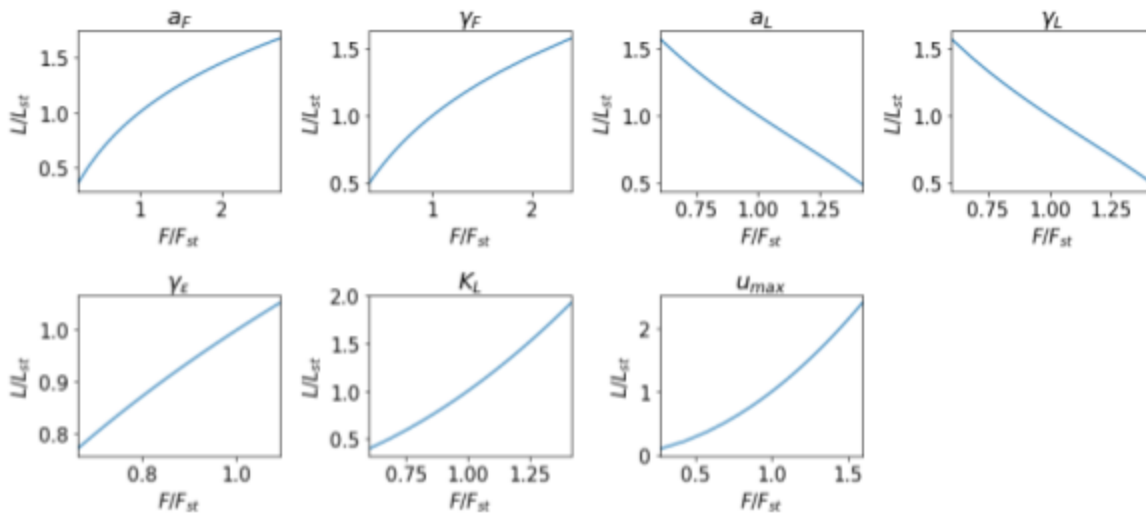Fig. 11 Fat and Leptin sensitivity to 16-fold change in each parameter

From Wiki: Gastric bypass is surgery that helps you lose weight by changing how your stomach and small intestine handle the food you eat. After the surgery, your stomach will be smaller. You will feel full with less food. The food you eat will no longer go into some parts of your stomach and small intestine that absorbs food.

Basal metabolic rate drops with age:
The parameter that probably changes the most with age is basal metabolic rate (BMR). This corresponds to the parameter γ_E (a sum of BMR and the cost of activity and exercise). BMR is high in young children and drops with age over childhood. It is roughly constant in the three decades from age 20-50, and drops again at ages above 50 (Fig. 12). My 8 year old youngest daughter Carmel has a BMI of 14, and mine is 25. On a good day. In our 20s and 30s we may be under the impression that youth will last forever.
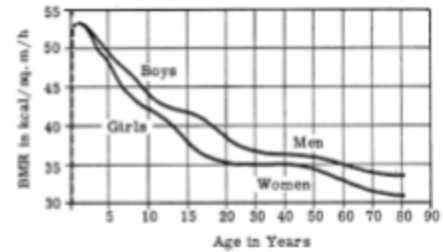

Fig. 12 BMR drops with age over childhood, and again at old age

Why did the feedback loop evolve? Current theory is that the leptin system serves an important evolutionary function, by protecting individuals from the risks associated with being too thin (starvation, infertility, poor immune function) or too obese (being eaten by predators). This hypothesis suggests that populations with low predation but high probability of famine and food insecurity (e.g. populations on small islands) will tend to accumulate genetic predisposition to obesity. Genetic predisposition collides with modernity, with its nutrition (high α_F, nearly unlimited access to food) and sedentary lifestyle (low γ_E), to generate the ongoing rise in childhood and adult obesity.

The take home message from these two lectures on weight is that graphical and math models, calibrated by experiments, can explain mysteries like the weight setpoint and how different interventions affect it.

Nice deep sigh of relief

References:

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- [Mechanisms of changes in basal metabolism during ageing](#) by CJK Henry 2000
- [A Nonadaptive Scenario Explaining the Genetic Predisposition to Obesity: The "Predation Release" Hypothesis](#) by John R.Speakman 2007

Appendix A: The Hill Function

Every biochemistry student learns to derive the Hill equation, named after Archibald Hill who used it in 1910 to describe oxygen binding to hemoglobin. Consider a receptor R binding n molecules of L with rate k_{on}, to form a complex [RLn], which falls apart at rate k_{off}. At steady-state the collisions of R with n molecules of L that make the complex, at rate k_{on} R L^n, are balanced by the complex falling apart, so that K_{on} R L= k_{off} [RLn]. Total receptor R_t concentration is a sum of free and bound R so that R+[RLn]= R_t. Putting this together yields $R = R_t/(1 + (L/K_L)^n)$ where K_L=k_{off}/k_{on} is the concentration of L at which half of R are bound, and n is the Hill coefficient.

Additional processes inside the cell affect the hormone action, including signal transduction pathways that convey the information form the cell membrane to its nucleus. Therefore, in our course we will use the Hill equation often, where we understand that K_L is not necessarily k_{off}/k_{on} but instead the concentration of hormone needed for a half-maximal effect on its target organ.

When the hormone causes an increase in physiological output, rather than a decrease, the Hill equation is $u/u_{max} = (L/K_L)^n/(1 + (L/K_L)^n)$

This function rises from zero when the input hormone is L=0, to a maximum of 1 at high L, reaching 1/2 when L=K_L. It can be derived by asking for the amount of bound receptors.

Exercise, lecture 1+2, hormone circuits:

1. Use the phase portrait to predict food intake and fat as a function of time in the following cases. The answer should be a schematic plot of u and F as a function of time, and a plot of the dynamics as arrows on the phase portrait):
    a) after a liposuction operation that removes some of an organism's fat.
    b) after a gastric bypass operation that reduces the stomach, modeled by reducing the maximal food intake u_{max}.
    c) after a drop in the rate of leptin clearance (removal) by the kidneys, γ_L.
    d) During hyperthyroidism, in which metabolic rate increases due to excessive levels of the thyroid hormones that control metabolism. Check your answer qualitatively by googling hyperthyroidism and seeing whether fat and appetite go up or down relative to normal (paste from internet, max 30 words).
    e) Think of an additional condition that is of interest. Which parameter(s) is affected?

2. Rodents provided with a variety of foods (buffet-style) eat more and gain fat compared to rodents provided with a single food type. Both can eat as much and as often as desired.
    a) What experiment can determine whether the food-fat conversion parameter α_F is the same in both cases? Explain using the phase portrait.
    b) Suppose the food-fat conversion parameter α_F is found to be the same in the buffet and single food experiments. What might be going on? (50 words).

3. Simulation of fat dynamics: this is our first taste of numerical simulations, which we will use in the course to understand hormone circuits. We start simple: A simulated animal has fat $F_0 = 1$ at time t=0 and then food supply stops so that food intake is u=0.

  a) Write an equation for dF/dt
  b) Numerically solve the equation, with $\gamma_E = 1$, $\gamma_F = 1$. Plot $F(t)$ versus time.
     (Be aware that in this simulation, it is normal for fat to drop below 0, even though in reality of course fat can not be negative)
  c) What is the value of Fst in this case? Show calculation.
  d) At what time does fat drop to zero? Answer either with the simulation or by an analytic solution of the equation.

Appendix:

Simulations by Omer (v=1/6h, fat turnover 3 days) agree reasonably with experiments (Jacqier, PLOS ONE 2014) on rat feeding: they show overshoot of eating after restricted feeding (green line), so that low fat after 'diet' increases food reward when diet is lifted.
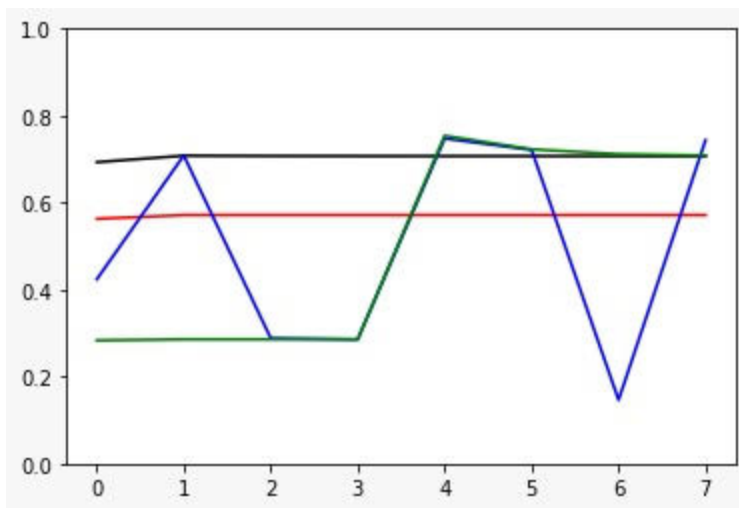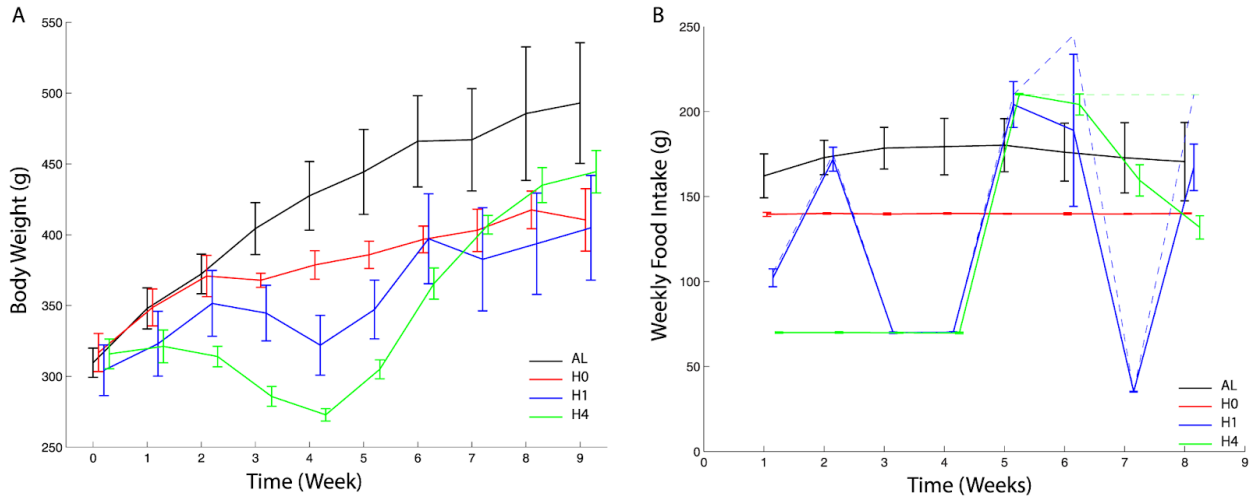
Where AL=ad libitum, H4= restricted feeding and then AL, H0= mild restriction, H1= time varying restriction.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
AL	Ad libitum							
H0	20 g	20 g	20 g	20 g	20 g	20 g	20 g	20 g
H1	15 g	25 g	10 g	10 g	30 g	35 g	5 g	30 g
H4	10 g	10 g	10 g	10 g	30 g	30 g	30 g	30 g
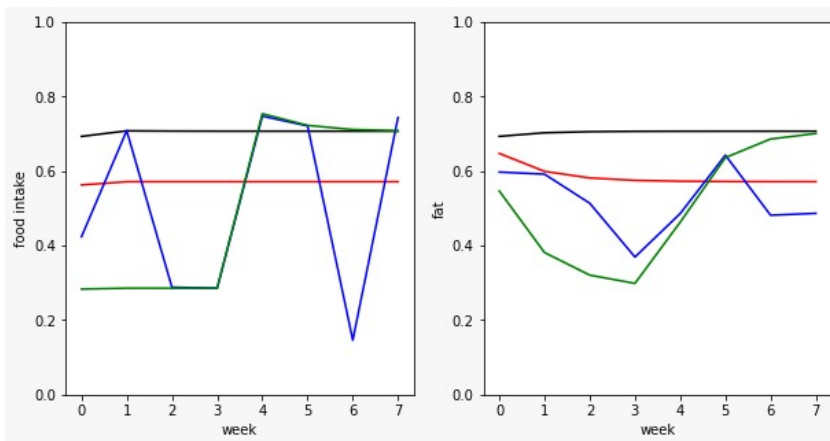
Model predicts:
1. Lower synthesis of leptin (as in ob/ob mice), corresponding to low α_L, results in higher levels of fat and food intake and lower leptin levels. Ob/ob mice indeed have much lower leptin levels and higher weight and food intake - what fold?

2. Lower affinity of leptin receptor (as in db/db mice), corresponding to higher K_L, results in higher levels of fat and food intake and higher leptin levels . This is observed, for example a 2.5-fold increase in weight in db/db mice corresponds to a 6-fold increase in serum leptin (where the model prediction is 2.5^2~6):
https://science.sciencemag.org/content/334/6059/1133/F3
https://jasn.asnjournals.org/content/15/3/645/tab-figures-data

3. Higher energy expenditure (as in voluntary wheel running), corresponding to higher γ_F, predicts a decrease of fat (by $\gamma_F^{-3/4}$) and an increase in food intake (by $\gamma_F^{1/4}$), as well as a decrease in leptin levels (by $\gamma_F^{-3/8}$). The Power laws are in the limit discussed above, in reality may be closer to -⅔, ⅓, 1/3. This agrees with experiments on wheel running in mice that report a 20% increase in food intake and 30% decrease in body fat (as expected in the model, $1.2^2 \cdot 0.7^{\tilde{}} = 1$ due to the ⅓ and ⅔ powers, relative fat decrease should be the inverse of relative intake increase squared):
   https://www.sciencedirect.com/science/article/pii/0031938482902116
   https://onlinelibrary.wiley.com/doi/full/10.1038/oby.2009.51F
   http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-879X2019000100301#B13
   Leptin also decreases (predicted to decrease by 20%, data?):
   https://diabetes-diabetesjournals-org.ezproxy.weizmann.ac.il/content/diabetes/46/7/1159.full.pdf
4. Leptin is proportional to the square root of steady-state fat percentage, given all other parameters equal: $L_{OPT}/F_{OPT}^2 = \frac{\gamma_F \alpha_L}{\gamma_L \alpha_F}$. This may explain the super-linear dependence between body fat percentage and serum leptin commonly observed in the clinic. [Figures]
5. The model also predicts a drop in food intake and fat when K_S decreases (by $K_S^{1/2}$), for example in bariatric surgery/ glp therapy.
6. Hyperthyroidism (change in energy balance nullcline) shows rise in u and drop in F. when treated there is no undershoot in appetite. In contrast, a diet which causes a rise in F, when stopped causes an undershoot in appetite. Thus, perturbing and relieving energy balance does not cause appetite overshoot, whereas perturbing and relieving diet or appetite nullcline does cause appetite overshoot.